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PB-265 250

Acute and Chronic Toxicity of Copper to the Fathead Minnow in a Surface Water of Variable Quality

Environmental Research Lab -Duluth, Cincinnati, Ohio

11 Feb 74

ERL-Duluth 0421

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. RE PB 265 250
EPA-600/J-76-008	JOURNAL ARTICLE	

4. TITLE AND SUBTITLE
Acute and Chronic Toxicity of Copper to the Fathead Minnow in a Surface Water of Variable Quality*

5. REPORT DATE

6. PERFORMING ORGANIZATION CODE
---

7. AUTHOR(S)
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8. PERFORMING ORGANIZATION REPORT NO.
---

9. PERFORMING ORGANIZATION NAME AND ADDRESS
Environmental Research Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
Duluth, MN 55804

10. PROGRAM ELEMENT NO.
1BA608

11. CONTRACT/GRANT NO.
---

12. SPONSORING AGENCY NAME AND ADDRESS
Environmental Research Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
Duluth, MN 55804

13. TYPE OF REPORT AND PERIOD COVERED
Journal article

14. SPONSORING AGENCY CODE
EPA/600/03

15. SUPPLEMENTARY NOTES *as published in Water Res. 10(1): 37-43.
**U.S. Environmental Protection Agency, Newtown Fish Toxicology Station, Cincinnati, OH 45244

16. ABSTRACT

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mg/l

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Aquatic Biology	Toxicity Test	06T
Toxicity	Chronic	06F
Fishes		06S
Water Pollution		
Copper		

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (This Report)	21. NO. OF PAGES
RELEASE TO PUBLIC	UNCLASSIFIED	9
	20. SECURITY CLASS (This page) UNCLASSIFIED	22. PRICE PC-A02-MF-A01

EPA Form 2220-1 (9-73)

EPA-600/J-76-008
JOURNAL ARTICLE

Water Research Vol. 10, pp. 37 to 43. Pergamon Press 1976. Printed in Great Britain

ACUTE AND CHRONIC TOXICITY OF COPPER TO THE FATHEAD MINNOW IN A SURFACE WATER OF VARIABLE QUALITY

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(*Received* 11 *February* 1974)

Abstract—Acute and chronic toxicity tests conducted with the fathead minnow and copper used as the source of dilution water a natural stream to which a sewage treatment plant upstream contributed a variety of materials known to affect acute copper toxicity. Nominal total copper 96-h median tolerance limit values (96-h TL50), determined with static testing procedures, ranged from 1.6 to 21 mg l^{-1}. Dissolved copper 96-h TL50 values ranged from 0.60 to 0.98 mg l^{-1}. The maximum acceptable toxicant concentration (MATC) based on survival, growth, reproduction, and hatchability of eggs was between 0.066 and 0.118 mg l^{-1}.

INTRODUCTION

Determining water quality criteria for aquatic life is like most biological problems in that experimental data are obtained under controlled laboratory conditions in order to predict effects that might occur under natural conditions. Mount (1968) and Mount and Stephan (1969) conducted experiments to determine the acute and chronic toxicity of copper to the fathead minnow (*Pimephales promelas* Rafinesque) in hard and soft water. The dilution waters used in these tests were controlled to maintain nearly constant hardness, pH, and alkalinity and essentially no suspended solids. In addition, temperature was maintained within a narrow, optimum range. Results of these tests indicated that hardness, or other characteristics such as pH, acidity, and alkalinity, affected both the acute and chronic toxicity of copper.

Environmental factors that influence acute copper toxicity are many and well documented. McKee and Wolf (1963) and Doudoroff and Katz (1953) in their summaries of copper toxicity mention dissolved oxygen, temperature, hardness, turbidity, carbon dioxide, magnesium salts, and phosphates. Organic compounds (Bender, Matson and Jordan, 1970), nitrilotriacetic acid (NTA) (Sprague, 1968), and spent sulfite liquor (Wilson, 1972) also are known to affect the acute toxicity of copper. Pickering and Henderson (1966) found a 60× and 15× difference in acute toxicity of copper in hard and soft water (in static tests) to the fathead minnow and the bluegill (*Lepomis macrochirus* Rafinesque), respectively.

The purpose of this study was to conduct acute and chronic toxicity tests at a streamside location in which a surface water of variable quality was used as the dilution water in order to collect information about the usefulness of such toxicity tests and the application factor concept, as defined by Mount and Stephan (1967), for estimating water quality criteria.

Twenty-one static acute mortality tests were conducted during this period to determine the acute toxicity of the copper to the fathead minnow in water from Shayler Run at different times during the chronic test. The chronic test described here studied the effect of several constant concentrations of copper on the survival, growth, reproduction, and egg hatchability of the fathead minnow; the procedures used were essentially the same as those described by Mount (1968) and Mount and Stephan (1969).

METHODS

The dilution water for the test was pumped continuously from a natural stream which received secondary waste treatment plant effluent. During the summer months most of the stream flow could be attributed to the plant discharge 3–4 miles upstream from the study location, and recovery of the stream was sufficient so that a varied natural flora and fauna existed at the point at which water was removed from the stream for the toxicity tests.

A temporary laboratory facility was constructed adjacent to the stream. Water temperature was not controlled, but a propane gas furnace provided sufficient heat to the building to prevent freezing.

Static procedures were chosen for the acute tests since the toxicity of copper in the stream water would vary during continuous-flow tests. Up to seven concentrations and a control were used in duplicate 19-l. bottles each containing 10 l. of stream water. These bottles were immersed in a water bath receiving stream water continuously so that the temperature of the test was maintained within 1–2°C of the temperature of the stream. Five fish were added randomly to each of the duplicate unaerated static test bottles for each test just after water samples were collected for copper analysis. The size range of fish for all tests was 20–69 mm total length. Dead fish were removed daily, and 96-h TL50 values were calculated by graphical interpolation (American Public Health Association, 1971).

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A proportional diluter (Mount and Brungs, 1967) was used for the chronic test to provide six nominal copper concentrations (0.60, 0.35, 0.20, 0.12, 0.06 and 0.03 mg l^{-1}) and a control. The flow for each concentration was split to duplicate (A and B), randomly arranged test chambers, 30 × 60 × 30 cm high. Water depth was maintained at 15 cm providing an approximate volume of 30 l. in each of the 14 chambers. The flow rate through the diluter was such that 190 l. were added to each chamber every day. A Mariotte bottle was used with the toxicant metering system and contained the aqueous stock solution of reagent grade copper sulfate ($CuSO_4 \cdot 5H_2O$).

A timer-controlled photoperiod was increased in hourly increments from 10 h at the beginning of the test in January to 16 h during the beginning of the spawning season and decreased to 14 h in September at the end of the 9-month exposure. This approximated the natural light pattern for southern Ohio. Aeration was provided periodically when there was a pump failure or an anticipated failure such as when high stream flows threatened to clog the pump intake with detritus.

Test fish for the chronic and acute toxicity tests were obtained from ponds at the Newtown Fish Farm, an Ohio Division of Wildlife Hatchery. The immature fish for the chronic test (20 per chamber) were collected in December, at which time they were approximately 5 months old and 32–38 mm in total length. After prophylactic treatment with formalin (20 mg l^{-1}) and a fungicide (Dexon*, 30 mg l^{-1}), and 2 weeks of observation, exposure of the fish to copper began in January 1970. When bacterial and fungal infections were observed the fish were treated with tetracycline hydrochloride and Dexon during the chronic test. All fish were treated similarly. The turbidity of the test water prohibited close observation of the fish. Fungus was especially a problem and was probably related to the high amount of organic material from the sewage treatment plant. At various times fish for the acute toxicity tests were also collected from the hatchery ponds and held in stream water at stream temperature for at least 2 weeks before testing.

All chronic test fish and fish being held for the acute toxicity tests were fed a dry trout food without antibiotics. This was supplemented by weekly feeding of live cladocerans. Some microcrustaceans entered the test chambers and holding tanks with the water supply.

Territorial and spawning sites were provided for the fish by placing six 7.6 × 7.6 cm half-tiles made of cement and asbestos in each chamber. Fathead minnows typically spawn on the underside of the tiles after which the female is driven from the area by the male who guards the eggs. The monolayer batch of eggs was removed from the tile into a Petri dish containing water from the same test chamber. The eggs were separated by wedging them apart with a scalpel and counted. Samples of 50 eggs each from seven spawnings were used to determine hatchability for each chamber in which spawning occurred. Eggs, 2–6 h-old, were placed in hatching cups attached to a rocker arm oscillating twice per minute. They were always hatched in the same concentration at which they were spawned, but all were hatched in one set of the duplicate chambers. Hatching usually required 4–6 days and depended on water temperature. No fry growth or survival tests were conducted, since earlier studies (Mount, 1968; Mount and Stephan, 1969) showed no effects on fry at concentrations of copper in which spawning was unaffected.

At the end of the 9-month test the adult fish were removed and anesthetized; live weight, length, and sex were determined.

Characteristics of the water were determined according to the American Public Health Association (1971). Dissolved oxygen, pH, and alkalinity were measured in each set of duplicate chambers once every 2 weeks, and hardness was measured less frequently. In addition, on each weekday hardness, dissolved oxygen, pH, and alkalinity were measured in the dilution water. These were measured on the first and last days of the static tests. Additional but less frequent determinations were made on a variety of other water quality characteristics.

One hour after copper was added to the static test containers, water samples were removed from one set of duplicates and filtered through a 0.45-μm membrane filter and acidified to determine dissolved copper.

Water samples for copper analyses were collected from the chronic study and composited for 7 days. Each set of duplicate chambers was sampled on alternate weeks. After acidification these samples were analyzed for total copper by use of atomic adsorption spectrophotometric procedures (Brungs, Leonard and McKim, 1973). Duplicate samples of water from the chambers containing a nominal copper concentration of 0.12 mg l^{-1} were removed weekly; total and dissolved copper were determined.

RESULTS

Analytical

Characteristics of the water in the acute and chronic test chambers are summarized in Tables 1 and 5, respectively. No measured dissolved oxygen concentrations in the chronic test chambers were below 5.0 mg l^{-1}. For the dilution water (Table 2) hardness varied from 88 to 352 mg l^{-1} (as $CaCO_3$), alkalinity from 56 to 248 mg l^{-1} (as $CaCO_3$), pH from 7.5 to 8.5, and dissolved oxygen from 5.0 to 13.0 mg l^{-1}. Additional but less frequent determinations of water quality are summarized in Table 3. These results are characteristic of enrichment from the sewage treatment plant effluent.

Total copper concentrations in the chronic test chambers are listed in Table 4, and these values will be used in the discussion of chronic test results. The mean concentration of dissolved copper in the nominal 0.12 mg l^{-1} chronic test concentration was 87% of the total (range was 66–104%).

Acute mortality tests

The nominal total copper 96-h TL50 values were based on the amount of copper added to the test bottles, and the dissolved copper 96-h TL50 values

Table 1. Means and ranges of chemical characteristics of water in test chambers*

Characteristic†	Number of analyses	Mean	Standard deviation	Range
Dissolved oxygen (mg l^{-1})	251	8.4	2.0	5.0–12.4
pH	245	--	--	7.6–8.6
Alkalinity (mg l^{-1} as $CaCO_3$)	243	183	37	76–244
Hardness (mg l^{-1} as $CaCO_3$)	35	274	47	148–340

*Analyses conducted in each test chamber every 2 weeks.

†Results of determinations of acidity ranged from 0.5 mg l^{-1} as $CaCO_3$ with 50 per cent of the results less than 1.0 mg l^{-1} as $CaCO_3$.

* Mention of trade name does not constitute endorsement by the U.S. Environmental Protection Agency.

Table 2. Monthly means and ranges of chemical characteristics of dilution water used for chronic study*†

Characteristic		January	February	March	April	May	June	July	August	September	Nine month period
Dissolved oxygen (mg l^{-1})	Mean	12.6	12.4	11.3	10.2	8.8	7.0	6.9	6.7	5.9	9.0
	Range	12.0–12.9	10.5–13.0	8.0–12.9	8.6–12.1	7.0–10.2	6.0–7.4	5.9–8.0	5.8–7.8	5.4–6.3	5.0–13.0
	N	10	17	18	19	17	19	23	18	10	151
pH	Mean	--	--	--	--	--	--	--	--	--	--
	Range	7.7–8.0	7.7–8.0	7.5–8.0	7.6–8.3	8.0–8.4	8.0–8.4	7.9–8.4	8.1–8.4	8.1–8.5	7.5–8.5
	N	10	17	18	18	17	19	24	18	11	152
Alkalinity (mg l^{-1} as $CaCO_3$)	Mean	204	152	143	155	189	212	192	184	211	181
	Range	168–248	78–204	92–192	96–198	96–220	160–228	88–232	90–220	184–220	56–248
	N	10	17	18	18	17	19	24	17	11	151
Hardness (mg l^{-1} as $CaCO_3$)	Mean	309	244	226	226	275	307	278	259	321	267
	Range	264–350	168–316	160–288	88–302	148–320	198–352	144–336	112–310	300–350	88–352
	N	10	17	18	20	17	19	24	18	11	154

*Results of nearly daily analyses between 9:00 and 10:00 A.M.

†Results of determinations of acidity ranged from 0–5 mg l^{-1} as $CaCO_3$ with 60 per cent of the results less than 1.0 mg l^{-1}.

were based on concentrations of copper in the filtrate after filtration through a 0.45-μm filter (Table 5).

Unfortunately, no dissolved copper measurements were made on nominal concentrations in excess of 16 mg l^{-1}. Therefore, when nominal total copper TL50 values exceeded 16 mg l^{-1}, no dissolved copper TL50 value is reported.

The range of nominal total copper TL50 values was from 1.6 to 21 mg l^{-1}. The dissolved copper TL50 values were much less variable (0.60–0.98 mg l^{-1}), despite a wide range in hardness (120–336 mg l^{-1} as $CaCO_3$), alkalinity (90–230 mg l^{-1} as $CaCO_3$), pH (8.0–8.5), temperature (2–30°C), other water quality characteristics and nominal total copper TL50 values (Table 5). Additional static tests were conducted after the chronic study described here was completed and produced nominal total copper 96-h TL50 values < 1.0–23 mg l^{-1} and dissolved copper 96-h TL50 values 0.52–1.0 mg l^{-1}.

Chronic test

Temperatures of the chronic test ranged from 0 to 30°C, with minimum and maximum weekly means of 5 and 26°C, respectively. The usual observed daily variation was between 3 and 10°C.

Within the first month of exposure 38 of the original 40 fish died at 0.56 mg l^{-1}. During the same period nearly 50% of the fish at 0.32 mg l^{-1} died. Eventually all fish died at 0.56 mg l^{-1}, and 75% died

Table 3. Means and ranges of various chemical characteristics of dilution water

Characteristic	Number of analyses	Mean (mg l^{-1})	Standard deviation	Range (mg l^{-1})
PO_4-P	23	3.4	1.6	0.5–6.6
Total P	23	3.5	1.6	0.8–6.6
Calcium	34	77	15	40–100
Magnesium	34	16	3.5	8–22
Total solids	36	458	121	250–792
Total dissolved solids	36	427	116	189–641
Boron	32	37	16	14–72
Potassium	32	6.2	2.8	2.3–14
Chloride	32	32	19	20–91
NO_3-N	25	2.5	1.2	<0.1–5.1
NO_2-N*	32	-	-	<0.1–0.5
NH_3-N*	29	-	-	<0.1–3.5
Conductivity (micromhos)	33	496	308	136–997
Total organic carbon	32	5.9	1.8	3.6–12
Organic nitrogen	26	0.62	0.19	0.1–1.1

*Most values were <0.1 mg l^{-1}.

Table 4. Measured total copper concentrations in chronic test chambers

Nominal copper concentration (mg l^{-1})	Chamber duplicate	Number of weekly composites analyzed	Mean measured copper concentration (mg l^{-1})	Standard deviation	Range (mg l^{-1})
0.60	A	16	0.59	± 0.033	0.49–0.61
	B	15	0.57	± 0.025	0.53–0.62
0.35	A	16	0.31	± 0.020	0.29–0.33
	B	15	0.32	± 0.018	0.29–0.35
0.20	A	16	0.18	± 0.014	0.16–0.21
	B	15	0.18	± 0.016	0.14–0.20
0.12	A	16	0.12	± 0.008	0.11–0.14
	B	15	0.12	± 0.010	0.10–0.14
0.060	A	16	0.064	± 0.006	0.053–0.074
	B	15	0.067	± 0.007	0.056–0.088
0.030	A	15	0.031	± 0.002	0.029–0.035
	B	15	0.035	± 0.005	0.029–0.050
Control	A	14	0.007	± 0.003	0.005–0.014
	B	14	0.007	± 0.002	0.004–0.011

Table 5. Summary of static bioassay results with fathead minnows

Test number	Date	Stream flow (cfs)	Hardness (mg l^{-1} as $CaCO_3$)	Alkalinity (mg l^{-1} as $CaCO_3$)	Temperature* range (° C)	Control pH	Nominal total copper 96-hr TL50 (mg l^{-1})	pH†	Dissolved copper 96-hr TL50 (mg l^{-1})
1	2/25	5.03	280	190	4-7	8.0	3.6	8.0	--
2	3/11	5.81	280	184	2-4	8.0	3.3	8.0	0.75
3	3/25	6.0	264	160	5-8	8.2	1.6	8.0	0.75
4	4/1		212	132	7-12	8.5	2.0	8.5	0.60
5	4/8	7.02	260	170	10-15	8.1	3.5	8.0	0.82
6	4/15	4.0	302	196	12-17	8.0	>16	-	--
7	4/22	5.62	224	160	15-21	8.0	9.7	8.0	0.98
8	4/30	11.3	228	158	17-23	8.1	5.0	7.9	0.83
9	5/13	33.1	150	96	19-24	8.1	2.8	7.9	0.93
10	5/20	2.57	340	-	16-21	8.2	11	7.3	0.87
11	6/10	1.46	294	210	22-25	8.0	16	7.0	0.73
12	7/1	0.75	308	210	22-30	8.3	16	7.2	0.88
13	7/8	0.30	136	230	20-27	8.3	21	7.3	--
14	7/16	1.37	280	180	20-23	8.4	12	7.5	0.77
15	7/23	1.03	280	180	24-27	8.2	11	7.3	0.63
16	7/29	0.95	266	170	22-27	8.4	20	7.4	--
17	8/5	0.72	310	204	22-23	8.3	21	-	--
18	8/10	20.6	120	98	20-22	8.3	2.2	8.0	0.69
19	8/19	0.88	310	204	20-25	8.4	21	7.9	--
20	8/26	1.06	298	200	21-24	8.3	16	7.5	0.86
21	9/9	0.92	324	220	18-24	8.3	20	7.5	--

*Test bottles were in a flow-through water bath with stream water to maintain test temperatures equal to those in the stream.

†These pH values were for water in test bottles containing copper concentrations on either side of the TL50 concentration. All other chemical characteristics are for control bottles.

Table 6. Spawning and egg production of fathead minnows at various copper concentrations

Mean measured copper concentration (mg l^{-1})	Duplicate	Number* of females during spawning	Number of males	Number of spawnings	Spawnings per female	Total eggs	Eggs per spawning	Eggs per female
0.007 (control)	A	9	2	46	5.1	7,576	162	842
0.007 (control)	B	9	2	74	8.2	13,755	186	1,528
0.031	A	3.5	4	38	10.9	5,218	137	1,491
0.033	B	10	2	69	6.9	9,533	138	953
0.064	A	9	1	60	6.7	7,632	127	848
0.067	B	3.25	2	52	6.3	5,125	99	621
0.12	A	6	7	1	0.2	8	8	1
0.12	B	10	5	13	1.3	1,578	121	158
0.18	A	9	3	0	-	-	-	-
0.16	B	3	1	0	-	-	-	-
0.31	A	3	0	0	-	-	-	-
0.32	B	1	3	0	-	-	-	-
0.56	A	1	0	0	-	-	-	-
0.57	B	0	0	0	-	-	-	-

*These numbers have been adjusted to account for those females that died during the spawning season.

at 0.32 mg l^{-1}. A second batch of fish was placed in 0.56 mg l^{-1}, and one fish survived several months until the end of the test. There was slight mortality due to copper at 0.18 mg l^{-1}. Fungal infections caused occasional fish mortality throughout the test, especially among the male fish. This mortality of males had been observeed in other chronic tests and is believed to be partially the result of competitive behavior associated with reproduction. Mortality early in the test was attributed to copper, as there was no difficulty with fungus at that time and the dead fish did not exhibit superficial evidence of bacterial or fungal infections.

The fish matured slowly during the winter and early spring as the result of the low test temperature, and spawning did not begin until 24 May 1970, 2 weeks after the photoperiod reached 16 h. The last spawning occurred on 24 August 1970, 3 weeks after the photoperiod was reduced to 14 h. At the same time the test temperature dropped and remained below optimum spawning conditions, and the test was ended on 15 September 1970.

Spawning was totally blocked at 0.18 mg l^{-1} and greatly reduced at 0.12 mg l^{-1} (Table 6), but duplication was insufficient to demonstrate significant differences related to copper concentration with analysis of variance tests. There was no effect on hatchability at any concentration at which eggs were produced. The mean hatchability for each concentration ranged from 88 to 94%.

Partial or total mortality at the high copper concentrations and loss of males by fungus resulted in small numbers of fish at the end of the test. Therefore no statement can be made concerning the effect of copper on growth during the test.

DISCUSSION

During this study the acute toxicity of total copper varied over a wide range, 1.6–21 mg l^{-1}, as water quality characteristics varied with season and flow. These results typify the classical condition of the effect of hardness and other characteristics on metal toxicity. However, the dissolved copper 96-h TL50 values were much less variable, 0.60–0.98 mg l^{-1}. It appears from these results that acute toxicity is coincidentally related to dissolved copper and that water quality characteristics that affect total copper toxicity in effect only determine the amount of total copper that has to be added to any particular water to elevate concentrations of toxic copper forms to lethal concentrations. This would probably not be the case in the presence of complexing agents such as EDTA that would permit the non-toxic complexed copper to pass through the filter. As greater amounts of these toxicity inhibiting materials occur, more copper must be added to have a comparable amount in a toxic form. This results in higher total copper TL50 values. Trama (1954) concluded that the acute toxicity of copper to bluegills depends on the amount of free copper in the water and that this amount is governed by the pH of the water; as the pH increases above 5.3 the copper salts precipitate.

The maximum acceptable toxicant concentration [MATC as described by Mount and Stephan (1967)] is in the range between a safe and unsafe concentration. Obviously, the mean egg production at 0.12 mg l^{-1} (80 eggs/female) is unsafe when compared to the control and low test concentration (1185 and 1222 eggs/female, respectively). However, at 0.066 mg l^{-1}, the mean was 735 eggs/female, a result intermediate to the others. Any assignment of safe or unsafe to this concentration would be arbitrary with no statistical confirmation, and the choice would have to be made on the basis of the authors' experience. The observed variability in egg production during numerous other chronic tests with the fathead minnow would indicate that 0.066 mg l^{-1} could not be called unsafe even though the egg production would appear to be marginal. Consequently, the MATC would probably be much closer to 0.066 than to 0.12 mg l^{-1}.

In contrast to the acute tests nearly all of the copper in the chronic test was dissolved (mean 87%; range 66–104%). Apparently at low copper concentrations, or in water low in toxicity-inhibiting characteristics, most of the copper added contributes to the effect on aquatic organisms. There still appears to be a slight dependence on water quality for the MATC for total copper and fathead minnows. In this study, at a mean hardness of 267 mg l^{-1} (as $CaCO_3$), the MATC was between 0.066 and 0.12 mg l^{-1}. Mount (1968) determined the MATC to be between 0.015 and 0.033 mg l^{-1} at a constant hardness of 200 mg l^{-1} (as $CaCO_3$). The MATC in water of a constant hardness of 30 mg l^{-1} (as $CaCO_3$) was between 0.011 and 0.018 mg l^{-1} (Mount and Stephan, 1969). This apparent variation in MATC with water quality is slight compared to variation in the 96-h TL50 values, and the use of a filter finer than 0.45 μm to define "solubility" might even eliminate this variability.

The application factor defined by Mount and Stephan (1967) is the MATC divided by the 96-h TL50. Under the usual controlled test conditions with a constant water quality the acute toxicity is relatively constant, and the calculation of an application factor is not involved. The variable acute toxicity results of this study require a more complex consideration. If a total copper 96-h TL50 value is to be used to calculate an application factor, many possibilities exist; among them are the mean, maximum, or minimum value. There are justifications for using, or not using, any of these three. The most important consideration is the fact that as total copper concentrations increase, an increasingly smaller percentage is dissolved and therefore available, apparently, to contribute toward a toxic response. The minimum acute toxicity (high 96-h TL50) values for total copper reflect a very low percentage of dissolved copper (about 5%) and

reduced toxicity. Consequently, the use of minimum or mean toxicity values would be less valid than the use of maximum toxicity (low 96-h TL50) values because most of the added copper is nontoxic (precipitated). The three low values (Table 5) were 1.6, 2.0, and 2.2 mg l^{-1}; at these values approximately 35% is dissolved. If an estimate of 1.6 mg l^{-1} is used to calculate the application factor for total copper and fathead minnows in this water of variable quality, the application factor would be between 0.066 ÷ 1.6 mg l^{-1} and 0.12 ÷ 1.6 mg l^{-1}, or 0.04 and 0.07. When a dissolved copper application factor is calculated by using the mean dissolved copper 96-h TL50 value (0.79 mg l^{-1}) and the safe and unsafe chronic dissolved copper concentrations (0.057 and 0.10 mg l^{-1}) this application factor would be between 0.07 and 0.13.

The problem of selecting appropriate acute toxicity values for estimation of an application factor, when precipitation or complexation occurs, is not unique to copper. Davies and Everhart (1973) concluded that when analyses of free or dissolved lead were used, the application factor succeeded in estimating MATC levels in both hard and soft water experiments. On a total lead basis, the use of an application factor for estimating the MATC, when a chronic test cannot be conducted, failed. Eaton (1974) also concluded that precipitation of cadmium at higher concentrations would reduce the usefulness of the application factor concept to estimate safe concentrations when hard water or resistant species are used to determine acutely lethal concentrations.

In addition to the studies with fathead minnows, chronic toxicity tests with copper and brook trout (McKim and Benoit, 1971) and bluegill (Benoit, 1975) have been conducted. The larval stage of the bluegill was the most sensitive stage according to Benoit (1975), however, he transferred control larvae to the various copper concentrations after the larvae produced in those concentrations failed to survive for reasons other than copper effects. The MATC values and estimated application factors for these species and the fathead minnow are summarized in Table 7. When the inherent biological variability is considered, together with the fact that total copper analyses were used for calculations, the consistency of the application factors is good. If all calculations were based on dissolved concentrations, the agreement would probably improve.

Table 7. Maximum acceptable toxicant concentrations and application factors for three fish species in three different waters

Species	Type of water	MATC (mg l^{-1}) safe	MATC (mg l^{-1}) unsafe	Application factor safe	Application factor unsafe	References
Fathead	Hard	0.015	0.033	0.03	0.07	Mount, 1968
Fathead minnow	Soft	0.011	0.018	0.14	0.24	Mount and Stephan, 1969
Brook trout	Soft	0.010	0.017	0.10	0.17	McKim and Benoit, 1971
Bluegill	Soft	0.021	0.040	0.02	0.04	Benoit, 1975
Fathead minnow	Variable quality	0.066*	0.120	0.04*	0.07*	--
		0.057†	0.10†	0.07†	0.13†	--

*Total copper.

†Dissolved copper.

The results of this and the cited studies would indicate that, for several of the heavy metals, toxicity should be based on dissolved quantities determined with 0.45 μm filter until a more accurate procedure is known to determine toxic metals directly or by extrapolation. Consequently, the use of the application factor (Mount and Stephan, 1967) to estimate safe concentrations of metals for aquatic species by using only acute toxicity data (based on total metal) would be limited whenever precipitation or some other toxicity-reducing condition occurs. If dissolved metal concentrations were used to calculate acute mortality results and application factors, the predictive ability of the factors apparently would improve.

Acknowledgements—The authors appreciate the assistance of the staff of the Newtown Fish Toxicology Station in the conduct of this study and reviews of the manuscript. Mr. Robert Williams of the Robert A. Taft Water Research Laboratory, Cincinnati, Ohio, performed most of the analyses in Table 3.

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